DESCRIPTION OF PLATE.

Fig. 1.—Microphotograph of Cortex of Dog's Kidney at Rest. ×500.
Fig. 2.—Microphotograph of Cortex of Opposite Kidney after Activity. ×500.
Fig. 3.—Cat's Kidney. Drawing of glomerulus and tubules after activity, showing dilatation of neck of tubule. ×500.

The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy and Germination of Seeds.—Part II.

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CONTENTS.

Introduction.

In the first part of this paper the influence of carbon dioxide in inhibiting the germination of moist seeds was described. The results obtained are summarised on pp. 623–625 of this paper.

In the present paper the relation of this inhibitory effect of carbon dioxide to temperature and oxygen supply is first to be examined, and then will be studied further narcotic or inhibitory effects of CO_2 as

exemplified in the natural inhibition of maturing seeds in the ovary, and the artificial prolongation of the dormant life of seeds which cannot survive naturally unless germination occurs soon after ripening.

Section I.—*The Relation to Temperature of the Inhibitory Effect of Carbon Dioxide on Germination.*

A large number of experiments were conducted to determine this relation. *Brassica alba* seeds were used. The result would appear to establish the conclusion that at low temperatures inhibition is caused by very small pressures of CO_2, while conversely at high temperatures high pressures of CO_2 are necessary to maintain continued dormancy. It would seem probable that this relation to temperature is significant in natural seasonal conditions. The technique in these experiments was the same as before described, the details of each experiment in full are unnecessary and a summary of the results obtained is given. The usual retardation effects were observed throughout, but the numbers in the table indicate only the final total germination out of 20 seeds.

Table I.—Total Number of Germinations with 20 *Brassica alba* Seeds in various Percentages of CO_2 in Air at different Temperatures. Compiled from 43 experiments.

Percentage of CO_2	0	2	4	6	9	12	15	18	24	30	36	42
3° C.	18	2	0									
7°	20	—	—	1	0							
10°	19	—	—	18	16	5	0					
17°	20	20	20	20	18	12	3	2	0			
20°	20	20	20	20	20	20	—	17	4	3	2	0
25′	20	20	20	20	20	20	20	20	19	19	14	7

The temperatures 10° C., 20° C., and 25° C. were maintained accurately within a variation of 0·5° C. The temperature of 3° C. obtained with melting ice varied to the extent of 1° C. The other two, 7° and 17° C., are averages of outdoor and indoor winter temperatures. The experiments were continued till no more seeds germinated.

It is necessary to consider the possibility of these results being due not to a decrease with rising temperature in the effectiveness of pressures of CO_2 in causing inhibition, but to an increase of oxygen stimulus caused by an increased permeability of the testa under the action of higher temperatures.

The following series of experiments were therefore conducted with *Brassica alba* seeds from which the testas had been carefully removed.

In these experiments with bare embryos, it was difficult in the early

stages to tell by eye whether germination had begun or not. The bare embryos in the first stages of germination could not be differentiated, but had to be described as either all germinating or all not germinating. In order to bring the results given in the following Table more into relation with those formerly obtained, small figures have been inserted to express the relative condition of growth at the end of the experiment, and the delay in germination as compared with the controls.

Table II.—Total Number of Germinations with 20 Bare Embryos of *Brassica alba* Seeds in various Percentages of CO_2 at different Temperatures. Compiled from 14 experiments.

Percentages of CO_2	Control 0	5	10	20	30	40	50
5° C.	20	16	8				
16	20	20_{16}	20_{12}	20_{8}	20_{4}		
20	20	20_{18}	20_{16}	20_{12}	20_{8}	20_{4}	

Ten per cent. of oxygen was present in each case. The temperatures 16° C. and 20° C. were maintained accurately within a variation of 0·5° C. In the case of the experiments at 5° C. the temperature was less accurately controlled, being obtained by melting ice.

It will be seen from the foregoing Tables that a rise of temperature of 10° C. necessitates roughly the presence of three times as high a partial pressure of CO_2 to cause inhibition. Thus in Table I at 10° C. no germinations occurred with CO_2 pressures above 12 per cent., while at 20° C. germinations occur up to 36 per cent. Similarly at 7° C. no germinations occur above 6 per cent., while at 17° C. germination proceeds with pressures up to 18 per cent. It must be remembered that the actual partial pressures of CO_2 in the tissues of the embryos is probably higher, especially where the testa remains intact, than the values expressed in the tables for the partial pressures of CO_2 in the atmospheres used.

The result of this series of experiments, both with whole seeds and with bare embryos, thus clearly indicates that a rise in temperature necessitates an increase in the amount of CO_2 necessary to produce inhibition in the seeds of *Brassica alba*. Conversely, a fall in temperature reduces the necessary amount of CO_2 to cause inhibition.

This relation of carbon dioxide inhibition to temperature may be emphasised. In the case of drugs acting chemically on the protoplasm the expectation is that their action will be more effective at high than at low temperatures. Here with carbon dioxide the reverse result has been

obtained. The fact must be borne in mind that we are dealing in this case with a gaseous agent more soluble at low than at high temperatures. This implies that to maintain in solution in the tissues the same concentration of carbon dioxide at a low temperature as at a high temperature necessitates a greater partial pressure of CO_2 in the atmosphere at the low temperature. Further work upon this relation of carbon dioxide inhibition in seeds to temperature is needed.*

Section II.—*Relation to Oxygen Pressure of the Inhibitory Effect of Carbon Dioxide on Germination.*

The presence of the testa between the embryo and its gaseous environment, as a membrane, only permeable with some difficulty, will, as has been pointed out, cause (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the actual CO_2 pressure in the embryo tissues.

It has been shown by the removal of the testa that temperature has, nevertheless, a direct effect in determining the inhibitory value of a given pressure of CO_2.

The following experiments were made to determine whether a varying oxygen supply might not also influence the inhibitory action of carbon dioxide. A large number of experiments were conducted at the same temperature, but with varying pressures of oxygen and carbon dioxide in the atmospheres used.

The testas were not removed in these cases. With a given pressure of CO_2, the temperature being fixed throughout, no variation in permeability in the testa was looked for. It is possible that an increased oxygen supply may cause a corresponding increase in the actual CO_2 pressure in the embryo tissues. The results show, however, that for the main purpose of the experiments this possibility may be neglected, as it clearly appears that an increase of oxygen supply decreases the inhibitory value of any given pressure of CO_2, while correspondingly a decrease in oxygen supply intensifies it, so that with small amounts of oxygen very low percentages of CO_2 will induce complete inhibition.

* In a critical consideration of the actual pressures of CO_2 in the embryo tissues at any temperature we should have to take into account not only the external partial pressure of CO_2, but also the rate of CO_2 production in the tissues and the rate of the escape of this CO_2 from the tissues by diffusion. Roughly, in relation to different temperatures, these two processes tend to cancel one another, and their combined effect to give the same value at all temperatures. In the above experiments no account has, therefore, been taken of any change with temperature of the rate of CO_2 production in the tissues or of the rate of diffusion from the tissues.

Table III.—The Effect of Decreased Partial Pressures of Oxygen on Carbon Dioxide Inhibition in *Brassica alba* Seeds. Small amounts of CO_2 are sufficient to cause inhibition if little oxygen is present.

Atmosphere of oxygen, carbon dioxide, and nitrogen.		Germinations out of 20 seeds while in artificial atmospheres.				Proportion of remainder which finally germinated on removal to air.
Oxygen percentages.	Carbon dioxide percentages.	2nd day.	3rd day.	4th day.	5th day.	
per cent. 21 (air)	per cent. 0 (air)	18	20	20	20	
8	0	13	18	18	20	
8	1	13	19	19	20	
8	3	15	15	18	20	
8	6	—	12	13	16	All
4	0	6	18	18	19	All
4	1	—	1	1	3	All
4	6	—	—	—	0	All

Average temperature, 14° C.

It will be noticed from the preceding Table that the effect of decreasing the oxygen supply is to intensify the inhibitory action of carbon dioxide. Thus, with a decrease of oxygen to 8 per cent., the inhibitory effect produced by 6 per cent. of carbon dioxide is very marked. With a decrease of oxygen in the atmosphere to 4 per cent., complete inhibition is produced by 6 per cent. of carbon dioxide.

Here again it must be remarked that this relation may very likely be significant in many cases of delayed germination under the influence of CO_2 in natural conditions.

Table IV.—Effect of various Partial Pressures of Oxygen on the CO_2 Inhibition of Germination in *Brassica alba* Seeds. Total number of germinations obtained out of 20 seeds.

Percentages of CO_3	0	9	12	15	18	21	24	27	30
Oxygen 5 per cent.	20	6	4	0	0	0	0	0	0
„ 10 „	20	18	17	10	3	1	0	0	0
„ 15 „	20	20	20	15	10	2	0	0	0
„ 20 „	20	20	20	18	12	—	3	0	0
„ 30 „	20	20	20	20	—	11	5	2	1

Average temperature 16·7° C.; extremes 13–18° C. The atmospheric residuum is N_2 in these experiments.

It will be seen that the amount of oxygen present has a definite effect upon inhibition by CO_2. Where there is only a pressure of 5 per cent. oxygen, complete inhibition is obtained by 15 per cent. CO_2, but, with 30 per cent. oxygen present, as much as 30 per cent. CO_2 is scarcely sufficient at the temperature used to cause inhibition.

The result of these experiments, therefore, indicates that a rise in the partial pressure of oxygen within the limits experimented on necessitates an increase in the amount of CO_2 necessary to produce inhibition in the seeds of *Brassica alba.* Conversely, a fall in the partial pressure of oxygen reduces the necessary amount of CO_2 to cause inhibition.

Section III.—*Carbon Dioxide as a Factor in the Dormancy of the Maturing Seed on the Plant.*

(a) *Arrested Development of Maturing Seeds not due to Lack of Moisture—Retarding Influence of the Testa.*—The maturation of the seed in normal conditions has certain features upon which it is desirable to dwell briefly.

The growth of the embryo proceeds continuously after fertilisation. In some cases it quickly reaches an advanced stage, and the radicle, plumule, and cotyledons may be formed very early. This growth, moreover, appears to resemble in some respects the growth which takes place subsequently, after germination, but in others it has the appearance of partial inhibition, the radicle apparently being not free to sprout as in germination. This appearance of inhibition increases in the cases of most seeds, until at the stage of complete maturation growth is apparently arrested or suspended. That there is some restraining cause tending to prevent growth present in the seed during the series of changes which is producing maturation may be proved, as in the experiments following, by the fact that the embryo, often at a comparatively early stage, though the seed be far from ripe, can be caused to sprout if removed to air.

The following experiments were conducted in order to show that neither lack of water nor any physiological insufficiency in the embryo can be considered as the cause preventing the still maturing embryos of beans and peas from sprouting, and so becoming cases of viviparity:—

(1) Two lots, 10 peas and 10 beans, were taken from pods which were still perfectly green and hardly yet fully swelled. These two lots were set to germinate at 20° C. on damp sand, with the result that all the seeds germinated perfectly. From these experiments it is clear that, in the case of the bean (*Vicia faba*) and of the pea (*Pisum sativum*), for some considerable period before the natural drying process commences, and while the growth of the pods is continuing, the seeds, if removed and placed in

germinating conditions, are capable of immediate germination. In this and similar experiments it was noticeable, especially in the case of peas, that removal of the testa greatly increases the rate of this germination. The following experiment was typical:—

Table V.—Increased Rate of Germination in Maturing Seeds of Peas when Testa is removed.

Description of seed.	Germinations.		Remarks.
	3rd day.	8th day.	
Peas fresh from the pod (10 with testa)	0	4	The testas of the six not growing were removed on the 8th day. All these six then sprouted within two days.
Peas fresh from the pod (10 without testa)	3	8	

(2) Further experiments to test the power of the embryo of the ripening bean and pea, before drying has commenced, to grow without the addition of moisture, were necessary. To this end 10 bean embryos taken from seed in immature condition were placed in glass tubes closed at both ends with bored rubber corks. They were placed at such distances as to avoid contact with each other.

In six days the radicles of all had sprouted; similar results were obtained with embryos taken from immature pea seeds.

These experiments were repeated another year with confirmatory results.

The bare embryos germinate readily in the above conditions. In parallel experiments made with whole immature seeds, the presence of the testa still intact was found to retard sprouting constantly. This retarding effect of the testa was more marked in these cases where no water was added to the green seeds from the pods than in the experiments above, in which such seeds were germinated in the ordinary way on damp sand. In connection with this action of the testa it is of great interest to find that Guppy, in a recent book containing the results of a wide series of studies upon seeds, remarks that "it is noteworthy that the viviparous habit is associated with the absence of seed coats."

(3) Experiments with germinating beans after complete air-drying in the laboratory showed that, at the moment of sprouting, these seeds might actually contain less water than they did when originally removed from the pod. These experiments were conducted both with whole seeds and with

the embryo alone. The following are representative examples taken from a series of experiments:—

Table VI.—Showing that Beans germinated after Complete Air-drying may actually contain at the moment of sprouting less water than they did when originally removed from the fresh green pod in the last stages of maturation before drying on the plant had commenced.

	Original weight of 10 beans when removed from fresh pods before natural drying had commenced.	Weight of the same 10 beans at the moment of sprouting during germination on damp sand after complete air-drying.
	grm.	grm.
Whole seeds	27·7	22·6
,, ...	24·1	21·6
Embryos alone	22·0	20·1

From these experiments it would appear, therefore, that neither lack of water nor any physiological insufficiency in the embryo can be regarded as the factor limiting germination in the maturing seeds of peas and beans.

Finally, the action of the testa as a retarding influence on germination has to be noted. In addition to experiments already given with seed still immature, the following experiments were made with dried seeds:—

Table VII.—Retarding Influence of the Testa in Germination of Dried Seeds.

Description of seeds.	Water uptake after 24 hours in percentage of original dry weight.	Germinations. 1st day.	2nd day.	3rd day.	4th day.	5th day.	9th day.
Peas 8 days dried in air after removal from pod, temp. 18–14° C.—							
10 with testas	139	0	0	1	5		
10 without testas	112	0	0	5	7		
Dry beans—							
10 with testas	116	0	0	0	4	9	
10 without testas....................	119	0	1	3	8	10	
Dry beans—							
10 with testas	100	0	0	1	3	—	8
10 without testas....................	116	0	10	10	10	—	10

In the above Table the retarding influence of the testa in the germination of seeds after drying is well marked.

(b) *Direct Estimation of the* CO_2 *Content of Maturing and Germinating Seeds.*—An enquiry is strongly suggested as to how far the non-germination

of the maturing seed, while still upon the parent plant, may be due directly to CO_2 inhibition or narcosis. In order to obtain evidence here an endeavour was made to ascertain the actual CO_2 content of ripening seeds. The method adopted was suggested by Dr. F. F. Blackman, for whose advice and direction during these researches I am deeply indebted. The technique of this method for determining the amount of CO_2 present in the tissues of seeds was as follows:—

Two lots of material of equal weight were taken in each experiment. One lot was crushed to thin paste in a mortar and left exposed to the air for 40 to 80 minutes. It seemed from experiments that this time was sufficient to allow the escape of the CO_2 present in the tissue mash. A known quantity of baryta was then added and a titration made with HCl. The second parallel lot was crushed immediately under an equal quantity of baryta and a similar titration made.

The difference between these two readings invariably showed that more baryta had been neutralised where the tissues had been crushed immediately in contact with it than where the tissue was first exposed for some time to air after crushing to a mash. These differences were taken as roughly expressing the relative CO_2 contents of the tissues used in these experiments.

The results obtained in a series of experiments made by this method to ascertain the CO_2 content of maturing peas and beans from fresh green pods and of the same seed during its drying in laboratory air, are given in the following table:—

Table VIII.—The CO_2 Content of Maturing Peas (*Pisum sativum*) and Beans (*Vicia faba*) when removed fresh from the Green Pod and during the first few days of drying.

Description of seed.	Grammes of H_2O per 100 grm. of seed.	Cubic centimetres of CO_2 per 100 grm. of seed.	Ratio CO_2/H_2O in tissues of seed.
Peas fresh from the pod	50	54	108/100
Peas after 4 days in laboratory air	22	145	660/100
Beans fresh from pod	60	51	85/100
Beans after 1 day's drying in laboratory air	56	46	82/100
Beans after 4 days' drying in laboratory air	51	41	80/100

In comparison with the above results the following Table gives those obtained in a second series of experiments made to determine the CO_2

content of similar seed during ordinary germination on damp sand after complete air drying in the laboratory:—

Table IX.—The CO_2 Content of Beans (*Vicia faba*) and Peas (*Pisum sativum*) while germinating.

Description of seed.	Grammes of H_2O per 100 grm. of seed.	Cubic centimetres of CO_2 per 100 grm. of seed.	Ratio CO_2/H_2O in tissues of seed.	Growth.
Peas after 18 hrs. germinating	67	64	96/100	None
" " 25 "	67	41	61/100	None
" " 39 "	70	43	62/100	Sprouting
" " 64 "	70	39	55/100	"
" " 97 "	65	16	24/100	"
Beans after 24 hrs. germinating	58	20	34·5/100	"
Beans after 7 days in germinating conditions	—	—	41/100	"
Beans after 5 days without testas in germinating conditions ...	—	—	16·5/100	"

The experiments lead to the conclusion that in the maturing seed, in the case of beans and peas, the CO_2 content of the tissues is higher than that under which actually germination takes place. In short, so far as these experiments have gone, it would seem that where the CO_2 content of the tissues is above a certain point germination does not occur and that the CO_2 content must fall below this point before germination takes place.

Section IV.—*Contrast of Depressant Action of High Partial Pressures of Carbon Dioxide with Stimulatory Effect of Low Partial Pressures. Carbon Dioxide considered as a Narcotic Agent.*

From the experiments already described it is definitely shown that the phenomenon of non-germination induced in the seed by CO_2 is one of temporary inhibition resulting in a condition strikingly similar to that of narcosis. The interesting question therefore presents itself as to how far this depressant action of carbon dioxide can be regarded as true narcosis.

Looking back, in the first place, through the history of previous work, it has to be noticed that the following results have been recorded as to the effect of carbon dioxide on the growth activity of plants:—

De Saussure (3) in 1804 found that an atmosphere containing 8 per cent. CO_2 restrained the growth of peas. Montemartini (4) found that over 7 per cent. CO_2 depressed the growth activity in the roots of peas. Chapin (5) in 1902 confirmed this. Böhm (6), Dr. Drabble (7), Prof. Farmer (1), and

Brown and Escombe (2) have also conducted experiments tending to show the restraining effect of carbon dioxide on growth. Dr. Drabble and Miss Lake in 1905 demonstrated the stimulation effect of small partial pressures of CO_2, observing that the growth in the length of pea roots was more rapid in 4 per cent. CO_2 than in air and than in percentages greater than 7 per cent. of CO_2.

Here it will be observed that there are two classes of effects recorded; an effect of retardation by higher percentages, and an effect of stimulation by lower percentages of CO_2. The stimulatory effect of small doses is a general property of narcotic agents. A further series of experiments was therefore arranged to test the effect of CO_2 in various proportions below the inhibitory percentage on the germination and growth of *Brassica alba* and *Hordeum vulgare*. The results* obtained with *Brassica alba* are shown in the following Table.

Hordeum vulgare gave similar results.

Table X.—Results obtained in Growth of 10 White Mustard (*Brassica alba*) Seeds under increased Partial Pressures of CO_2, showing the Stimulatory Effects of Low Percentages, rising to a Maximum and then declining towards Inhibition.

Percentage of CO_2 in the atmosphere in each case.	Increase in weight expressed in percentages of original weight of seed.	Average length of growth at termination of experiment in centimetres.
0	23·3	3·8
2	16·0	4·0
3	34·0	4·4
4	23·0	4·3
5	9·0	3·5
10	8·0	2·0
(25 per cent. CO_2 gives complete inhibition)		

This experiment was conducted in a dark room. Average temperature, 16·5° C.

In the foregoing Table it will be observed that the first effect of carbon dioxide is one of stimulation in low percentages. This increases to a

* The rate of germination was not increased by the low percentages of CO_2 in this experiment, but as has been shown in the case of beans and peas in Table IX, the actual CO_2 content of the seeds is high and falls from an initially inhibitory value as germination proceeds. We should not expect, therefore, small doses of CO_2 in the atmosphere to have a marked stimulatory effect, if any, upon the rate of germination, though their effect upon growth after the escape of the initial high partial pressures of CO_2 in the seeds is clear.

maximum which, at the temperature used, is obtained at about 3 per cent. or slightly over, and then declines again through a restraining effect to complete inhibition.

We appear, therefore, to have in view results confirmatory of the hypothesis that we are here dealing with the effect on germination and growth of a true narcotic agent, and that the results induced by CO_2 in the resting seed are a phase of narcosis.

Section V.—*Influence of* CO_2 *in Enforcing Dormancy in Certain Seeds which do not Naturally have a Long Dormant Phase. Seeds of* Hevea brasiliensis.

A considerable amount of work has been done in the past—work which is well summarised by Becquerel (8)—upon various effects produced in dry seeds by sealing them in various gases and vapours, including CO_2. Becquerel discounts the value of part of this work on the ground that it has been conducted on seeds with impermeable testas, so that the gases used could not be considered to have reached the plant embryo.

In a number of experiments conducted during this inquiry on seeds with naturally permeable testas, and on rapidly deteriorating seeds in which the testas may be assumed to be at least partially permeable, carbon dioxide was found in nearly all cases to have certain definite effects, such as might have been expected from the foregoing experiments conducted upon wet seeds in germinating conditions.

The results of this work, which is still in progress, have not yet been correlated, but one aspect of them may be referred to here, as bearing directly upon the central problem discussed in this paper.

One of the most rapidly deteriorating seeds is that of *Hevea brasiliensis.* In planting in the tropics it is found that it is always desirable to put the seed in the ground within a fortnight, and Mr. C. Curtis, late director of the Botanical Gardens, Penang, from whom the seeds used were obtained, writes that even in such circumstances 70 per cent. germination is considered good. This rapid deterioration of the seed has been a difficulty in the recent extension of rubber plantations, and the question of the best conditions for preservation in packing and export has been an important one, leading to practical research. The seeds are at present usually packed in ground charcoal and ashes. Their size is about that of an average acorn or larger. They have easily permeable testas and a high water content, and while living they were found to be respiring very rapidly. They were also found to be very intolerant of drying. The seeds in the experiments considered in this research were enclosed in hermetically sealed flasks under various conditions,

and it was found as the outcome of a number of experiments that when they were sealed in the proportion of 40 to 50 seeds to 1200 c.c. of air the following results were obtained:—(1) A partial pressure of CO_2 of 40–45 per cent. was created in the flasks by the life processes of the seeds, and (2) there was a marked prolongation in their period of vitality.

In the following Table the results of two experiments are given. The imported seeds, when received in this country, were necessarily some weeks old. The temperature at which germination tests were conducted was 27° C. in a thermostat:—

Table XI.—Showing prolonged Dormancy of *Hevea brasiliensis* Seeds sealed in flasks as described. Flasks opened after 50 days. A test germination, begun at the time of receipt of the seeds, gave 40 per cent. germinations.

How kept during 50 days.	Analysis of atmosphere on opening flasks after 50 days.			Percentage of germinations after 60 days.
	CO_2.	O_2.	N_2.	
	per cent.	per cent.	per cent.	per cent.
Experiment 1—				
50 seeds in air in 1200 c.c. sealed flasks	45	1·3	53	40 (good plants)
50 seeds in air in 1200 c.c. open flask	—	—	—	8
50 seeds in air in commercial packing as sent from tropics	—	—	—	16
Experiment 2—				
20 seeds in air sealed in 500 c.c. flask	40	4·0	56	40 (good plants)
20 seeds in nitrogen sealed in 500 c.c. flask	41	1·0	58	25 "
20 seeds in air in 500 c.c. open flask	—	—	—	0

The first of the above experiments took place over the months of November and December. The flasks were kept in a temperature varying from 10° to 15° C.

The second experiment took place during December and January. The flasks were kept in the laboratory, the temperature varying from 18° to 13° C. There was considerable internal pressure when the flasks were opened in both experiments.

In a third experiment the period during which the seeds were kept from date of importation was prolonged to 90 days. The average temperature was considerably higher, the months over which the experiment extended being September, October, and November. In this case 10 per cent.

germinations were obtained with seeds sealed in air in the proportion mentioned against *nil* with seeds kept in commercial packing, *nil* with seeds kept in open air, and *nil* with seeds sealed in nitrogen.*

In the foregoing experiments it will be observed that large seeds enclosed in permeable seed coats and sealed with a definite proportion of air in an impenetrable outer envelope were being dealt with. In these conditions, where the life processes of the seeds resulted in the creation in the flask of a partial pressure of CO_2 of 40–45 per cent. the vitality of the seeds was markedly prolonged.

A conclusion which Becquerel reaches, as the result of his researches, is that in all cases of longevity in dry seeds the testas are exceptionally strong and impermeable. The problem of the dry seed enclosed in an impermeable or almost impermeable testa has certain striking affinities—in that gaseous exchange in either direction is hindered or prevented—to that of the wet seed, though in apparently good germinating conditions, which does not germinate. But with the former problem we are not at present directly concerned in this research.

Section VI.—*Biological Importance of Dormancy in Moist Seeds.*

The seed is a comparatively late arrival in geological time, and the perfecting of its function has of necessity been a great point in the struggle for existence amongst plants. A leading cause in the success of the Angiosperms, as Prof. Seward has pointed out, has consisted in the efficiency of the arrangements for nursing the embryo. There can be no doubt that a ruling factor in this efficiency has been the adjustment of all the life processes of the moist resting seed to the end of attaining a fit time for germination. It is suggested by these experiments that the presence of carbon dioxide in the tissues of the embryo acting as a restraining and inhibiting agent on the life processes of the seed, and as a dominant factor in relation to the oxygen stimulus, has been utilised in attaining this efficiency of the latent seed for which fit conditions of germination have not yet arrived. The various structures of the testa and its behaviour under different conditions in regulating the gaseous exchanges

* The favourable results obtained in these experiments in prolonging the vitality of these rapidly deteriorating seeds were greatly in excess of those which are secured by present commercial methods of packing for transport and import. In experiments on a large scale the seeds might be simply sealed (in the proportions of air mentioned) in large carboys, such as are used for the transport of distilled water, covered with wicker or wire netting. In case of too high an internal pressure, arising from overfilling with seeds, a simple form of safety valve might be inserted in the sealing.

appear to be important factors in obtaining the necessary adjustments to natural conditions.

Emphasis may properly be laid on the fact that it is these adjustments of the moist seed when in apparently suitable conditions of temperature, moisture, and oxygen supply, while awaiting the fit time for germination, and not so much the adjustments of the resting dry seed, that have formed the central problem of seed life in conditions of nature. The maintenance of latency when the moist seed is in conditions of medium temperature, oxygen supply, and moisture, has been the problem of the maturing seed on the parent plant. It has been the problem of a large proportion of native seeds which fall upon the ground in summer and autumn, but whose fit time for germination does not arrive till the following spring. It has, beyond doubt, been the problem also of many species of plants in the struggle for existence whose chances therein must have often been increased many-fold by the capacity of their seeds to lie dormant in the ground for indefinite periods, ready to resume activity with sporadic germination when suitable conditions arise such as, for instance, occurred in the case of the *Brassica alba* seeds of these experiments when the testas became dry or ruptured.

Section VII.—*Summary and Conclusions.*

Part I.—Experiments were conducted showing that the germination of seeds is retarded or inhibited by high partial pressures of CO_2 in the atmosphere. This retardation and inhibition produced by CO_2 was shown to be unaccompanied by injury. The seeds used in these experiments fall into two classes. In the first class the seeds germinated at once after removal from the inhibitory CO_2 pressures (beans, cabbage, barley, peas, onions). In the second class the inhibition continued indefinitely after the removal of the inhibitory CO_2 pressures, and is terminated only by complete drying (and rewetting), or by the removal of the testa. In this class a lowering of the permeability of the testa to gases under the influence of CO_2 is indicated, a change which would have two results: (1) a reduction in the amount of oxygen reaching the embryo; and (2) a relative rise in the actual CO_2 pressure in the embryo tissues. The condition of prolonged inhibition after removal to air produced in *Brassica alba* is strikingly suggestive of the condition of seeds often met with in nature, the germination of which is delayed in spite of suitable conditions of temperature and water. The results obtained in the laboratory with *Brassica alba* seeds were reproduced in the soil in natural conditions by CO_2 arising from decaying vegetable matter. The high CO_2 content of the soil air in these experiments was found to

continue for a considerable period. Attention was called to the importance of these facts in agriculture.

Part II.—A long series of experiments was carried out to determine the relation of carbon dioxide inhibition in seeds to temperature and to oxygen supply. Low temperatures and low oxygen supply were both found to increase the inhibitory value of given partial pressures of CO_2, while inversely the inhibitory value of given carbon dioxide pressures diminishes with a rise of temperature and with a rise of oxygen pressure. The probable relation of these facts to the dormancy of the moist seed in natural conditions was pointed out.

The arrested development of maturing seeds on the plant was shown not to be due to lack of moisture or to any physiological insufficiency. The seeds in this stage were shown to contain in their tissues more CO_2 than seeds normally germinating contain at the moment of sprouting. The presence of the testa was shown constantly to retard the germination both in seeds taken from the parent plant before natural drying and in seeds after complete drying and storing. Attention was drawn to the correlation found to exist between the viviparous habit and the absence of seed coats.

Carbon dioxide has been considered as a narcotic agent. Previous work on the action of CO_2 upon growth has been quoted. The stimulatory effect of low partial pressures, rising to a maximum with increasing pressures and then declining to inhibition with higher pressures of CO_2, has been demonstrated by experiments with *Brassica alba* and *Hordeum vulgare* germinated in the dark.

In the case of certain rapidly deteriorating seeds (*Hevea brasiliensis*) the carbon dioxide naturally produced by respiration of the seeds in a closed flask rose to 40 per cent. and the presence of this was found to be accompanied by a marked prolongation of vitality in the seeds. This prolonged vitality was far in excess of that reached with the present commercial method of packing these short-lived seeds for export.

When we correlate the results of these different lines of experiment we seem to get in various directions evidence of the importance of carbon dioxide pressure as a controlling influence in the biology of seeds. This influence may be formulated briefly in the following principles :—

(1) The resting stage of the moist seed is primarily a phase of narcosis induced by the action of carbon dioxide.

(2) Both the arrested development in the case of the moist maturing seed on the plant, and the widely occurring phenomenon of delayed germination in the case of the moist resting seed, which does not germinate although in apparently suitable conditions of temperature, moisture, and oxygen supply,

are related to an inhibitory partial pressure of carbon dioxide in the tissues of the embryo.

(3) Germination when it takes place is related to a lowering of the value of this inhibitory partial pressure of carbon dioxide in the tissues.

(4) The inhibitory value of a given carbon dioxide pressure diminishes with a rise of temperature.

(5) The inhibitory value of a given carbon dioxide pressure diminishes with a rise of oxygen pressure.

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